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Sutureless amniotic membrane transplantation for ocular surface reconstruction with a chemically defined bioadhesive

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ABSTRACT

The purpose of this study was to evaluate the efficiency and safety of a sutureless amniotic membrane transplantation (AMT) for ocular surface reconstruction with a chemically defined bioadhesive (CDB). The CDB was synthesized from aldehyded polysaccharides and ε-poly(L-lysine), two kinds of medical and food additives, as starting materials. Biocompatibility assay indicated that the CDB showed excellent biocompatibility with *in vitro* and *in vivo* ocular surface tissues and most of the CDB was histologically degraded within 4 weeks. Sutureless AMT using the CDB was safely and successfully performed onto a rabbit scleral surface. Transplanted amniotic membrane (AM) evaluated by histological, electron microscopic- and immunohistochemical examination indicated that the CDB did not affect normal differentiation of the cells or the integrity of the surrounding tissue. Thus, this newly developed CDB was found to be very useful for sutureless AMT for ocular surface reconstruction, without considering the risk of infection. It has the ability to fix AM to the ocular surface for a long time-period without additional inflammation, scarring, or damage to the surrounding tissues.

1. Introduction

Human amniotic membrane (AM) has been shown to possess various kinds of biological effects such as anti-inflammatory [1,2], antifibroblastic [3], anti-microbial [4], and anti-angiogenic properties [5], and promote epithelialization by facilitating the migration of epithelial cells [6], reinforcing adhesion of basal epithelial cells, and promoting epithelial differentiation [7]. It also produces growth factors that promote epithelial cell growth [8]. Due to these desirable properties, AM has been used as surgical material in a wide variety of operations. Since Kim and Tseng achieved successful corneal re-epithelialization in chemical burns by amniotic membrane transplantation (AMT) in 1995 [9], AM has been widely used in eyes with ocular surface diseases such as persistent corneal epithelial defects [10,11], pterygium [12], symblepharon [13,14], and stem cell deficiency [9,13,15–18].

AM is usually sutured onto the ocular surface using 10-0 nylon sutures to fix [19]. Although the suturing method makes for a secure attachment, it inflicts trauma to the ocular surface. Moreover, a prolonged operative time and technical skill are needed for effective suture placement. Sutures can not only cause postoperative pain and discomfort which is a significant problem for patients [20], but can also be associated with suture-related complications such as suture abscesses [21,22], granuloma formation [23], and tissue necrosis [24]. To solve these problems, sutureless techniques have been applied for various kinds of operations on the ocular surface including conjunctival closure after surgery [25,26], pterygium surgery [20,27,28], corneal stem cell transplantation [29,30], lamellar keratoplasty [31], and conjunctivochalasis [32].

Recently, we developed a sutureless AMT using bioadhesive-coated freeze-dried AM for ocular surface reconstruction [33]. This AM is based on our previous report regarding sterilized, freeze-dried AM [34]. Being coated by a minimum dose of fibrin glue, bioadhesive-coated freeze-dried AM can be readily used after opening the package. It can be kept sterilized at room temperature (RT) for long periods without deterioration so that it can easily be stored, transported, and used. This 'ready-to-use' AM has enabled AMT to be easily performed in every hospital in the world.

Although these newly developed sutureless methods using fibrin glue achieved successful outcomes, safety and logistical problems still remain. Some viruses, such as parvovirus B19 (HPV

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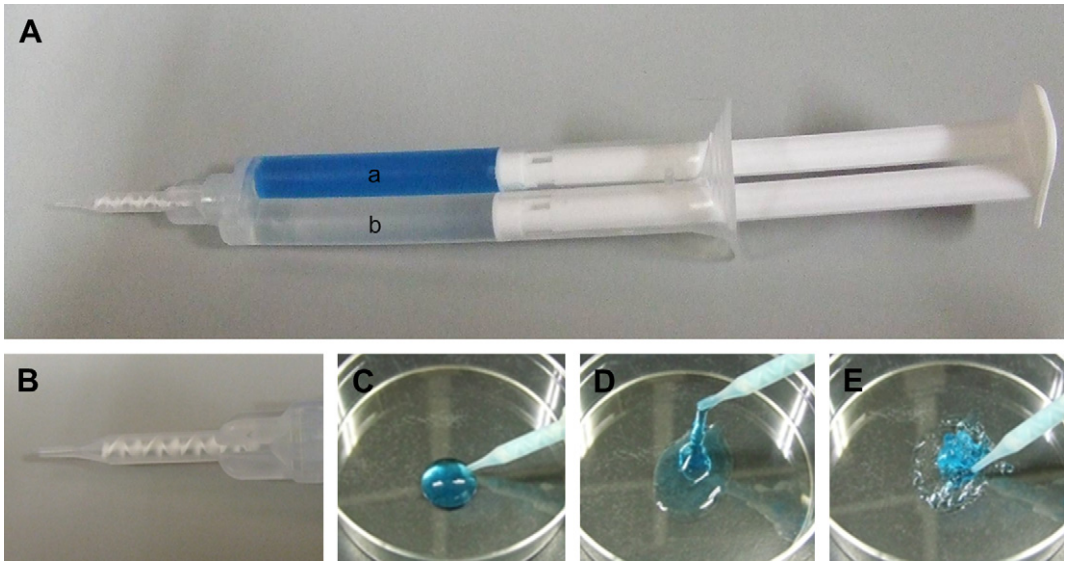

Fig. 1. (A) This glue prepared with syringe-like container with two cylinders (a, b); one cylinder (a) is filled with 2 ml of 14 w/w% aldehyded dextran solution with aldehyde introduced ratio of 0.43 per sugar unit, and the other (b) with 2 ml of 7 w/w% ε-poly(L-lysine) solution containing 2.1% w/w% acetic anhydride. The container has special mixing tip which can mix two solutions each other by equal volume as passing through it (B). The mixed adhesive is gradually gelatinized (C–E). The tip is disposable because the adhesive had been gelatinized in the tip before next application.

B19) are particularly difficult to totally remove or inactivate, and human infection has been reported after the use of fibrin glue that had been prepared in house from pooled plasma [35]. Moreover, in thoracic surgery, epidemiological evidence suggests that more than 20% of uninfected people were subsequently infected with HPV B19 by use of commercially available fibrin sealant during the procedure [36]. In addition, there is a potential for the transmission of prions originating even from commercially available human plasma [37]. Although the risks of both diseases are extremely low, patients should be informed before surgery. Some other tissue adhesives using collagen or gelatin of animal sources have been previously developed and experimentally evaluated, but like with fibrin glue, the risks of infection have still remained. If non-biologic and chemically defined bioadhesives can be successfully applied, it would prove ideal for safe and simple ocular surface reconstruction.

In this report, we present our newly developed CDB for sutureless AM transplantation. It was made from antibiotic food additives and characterized by its self-degradability, low toxicity, and stronger bonding property. To the best of our knowledge, there have been no reports investigating ocular surface reconstruction using safe and effective CDB.

2. Materials and methods

2.1. Preparation of CDB

The mechanism of CDB gelation is based on Schiff base formation between oxidized and aldehyded polysaccharides and ε-poly(L-lysine), two kinds of antibacterial additives for medicine or food [38]. CDB is prepared with a syringe-like container with two cylinders (Fig. 1A). One cylinder is filled with 2 ml of 14% w/w aldehyded dextran solution (molecular weight: 75K Da) with aldehyde introduced ratio of 0.43 per sugar unit, and the other with 2 ml of 7% w/w ε-poly(L-lysine) solution (molecular weight: 4K Da) containing 2.1% w/w acetic anhydride. CDB was used after filtration sterilization of both solutions using a syringe filter with 0.2 μm pore size. When the end of the syringe is pushed, the two solutions are mixed together in equal volumes as they pass through the tip (Fig. 1B) and then gradually gelatinize (Fig. 1C–E). Gel formation time, which can be altered by aldehyde introduction in dextran, was approximately 31 seconds at the temperature of 37 °C, counted by the same method as in the previous report [38]. Degradation speed can also be altered by acetic anhydride concentration in ε-poly(L-lysine). The CDB in this report was self-degradable within 4 days at the temperature of 37 °C *in vitro*.

2.2. Preparation of AM

AMs were prepared according to our previously reported method [39]. Briefly, after obtaining proper informed consent in accordance with the tenets of the Declaration of Helsinki, and with approval by the Institutional Review Board of Kyoto Prefectural University of Medicine, human AM was obtained under a sterile condition from seronegative donors after an elective caesarean section. The AM was washed with sterile phosphate buffered saline (PBS) (Nissui, Tokyo, Japan) containing antibiotics and antimycotics. The chorion was peeled off manually and epithelial cells were removed by incubation with 0.02% ethylene diamine tetra-acetic acid (EDTA) (Nacalai, Kyoto, Japan) at 37 °C for 2 h. Denuded AM was cut into approximately 4 × 4 cm pieces, and cryopreserved at −80 °C in a sterile vial containing Dulbecco's modified Eagle medium and glycerol in a volume ratio of 1:1. Before use, the AM was thawed by warming the vial to RT.

2.3. Examination of biocompatibility of CDB

Before using the CDB in this assay, we have already investigated each cytotoxicity test of aldehyded dextran and poly(L-lysine) using mouse established cell line L929 [38]. IC50 (sample concentration in culture medium which suppresses the cell viability down to 50%) of them was 6000 and >10,000 μg/ml, respectively. These values are 1000 times as much as those of formaldehyde and glutaraldehyde (1.7 and 3.9 μg/ml, respectively), which suggests quite the low toxicity of both components. Because the difficulty in cytotoxic evaluation of hydrogel materials *in vitro*, *in vivo* biocompatibility test of gelation sample was carried out. All experiments in this study were performed in accordance with the Committee for Animal Research at Kyoto Prefectural University of Medicine and according to the ARVO statement on the Use of Animals in Ophthalmic and Vision Research. To investigate the biocompatibility of CDB with the ocular surface, it was injected into the subconjunctival

Table 1
Antibodies and their sources

Specificity	Immunized	Dilution	Sources	Cat. number
Cytokeratin 1	Mouse, mAb	20×	Novocastra	NCL-CK1
Cytokeratin 4	Mouse, mAb	200×	Novocastra	NCL-CK4
Cytokeratin 10	Mouse, mAb	50×	Novocastra	NCL-CK10
Cytokeratin 13	Mouse, mAb	200×	Novocastra	NCL-CK13
MUC5AC	Mouse, mAb	200×	Zymed	18-2261